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Potentiometry and NMR Studies of 1,5,9-Triazacyclododecane-*N,N',N''*-triacetic Acid and Its Metal Ion Complexes

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A new chelating macrocycle, 1,5,9-triazacyclododecane-*N,N',N''*-triacetic acid (DOTRA), has been synthesized, and its complexes with Zn^{2+} , Cd^{2+} , Ca^{2+} , Mg^{2+} , and Mn^{2+} have been examined by potentiometry and NMR spectroscopy. The first protonation constant of the free ligand ($\log K_1 = 12.8$) was determined spectrophotometrically, while the remaining were evaluated from potentiometric data ($\log K_2 = 7.55$, $\log K_3 = 3.65$, $\log K_4 = 2.1$). DOTRA forms complexes with Mn^{2+} , Mg^{2+} , and Ca^{2+} rather slowly but reacts much more rapidly with Zn^{2+} and Cd^{2+} . As reported previously for the nine-membered-ring triaza analogue NOTA, DOTRA also forms a more stable complex with Mg^{2+} than with Ca^{2+} ($\log K_{\text{st}} = 7.1$ versus 6.0). High-resolution NMR spectra of the Zn^{2+} , Cd^{2+} , and Mg^{2+} complexes ($\log K_{\text{st}} = 19.0$, 15.7, and 7.1, respectively) indicate the three six-membered chelate rings are symmetrical and quite rigid in aqueous solution, as evidenced by nonequivalence of all six chelate ring protons.

Introduction

The complexation properties of the macrocyclic polyaza polycarboxylates have attracted considerable interest in recent years because the structural, equilibrium, and kinetic behavior of their metal ion complexes differ considerably from those of the analogous noncyclic polyamino polycarboxylates. Some of these ligands are also of practical importance, since their gadolinium(III) and manganese(II) complexes may be used as contrast agents for magnetic resonance imaging.^{1,2} While several tetraaza tetracarboxylates have been prepared and investigated, the only triaza tricarboxylate reported so far is 1,4,7-triazacyclononane-*N,N',N''*-triacetic acid (H_3NOTA).³ NOTA forms 1:1 complexes of high stability with a number of metal ions. Such complexes have been prepared with Al(III), Cr(III), Mn(II) and -(III), Fe(II) and -(III), Co(II) and -(III), Ni(II) and -(III), and Cu(II).^{4–7} NOTA was found to be coordinated as a hexadentate ligand, and contrary to the complexes of the flexible EDTA, which often contain a H_2O molecule in the inner sphere (e.g. Mn^{2+} -(EDTA)(H_2O)⁸), the NOTA complexes with these ions are all six-coordinate. The geometry of several complexes investigated by X-ray diffraction was found to be distorted octahedral^{5,7} ($\text{Cr}(\text{NOTA})$, $\text{Ni}(\text{NOTA})^-$) or distorted prismatic⁵ ($\text{Fe}(\text{NOTA})$, $\text{Cu}(\text{NOTA})^-$) with a significant strain in the molecular framework.

The stability constants of the NOTA complexes are in general similar to those of EDTA.³ A significant size selectivity was observed for the smaller Mg^{2+} ion, however. The stability constant of $\text{Mg}(\text{NOTA})^-$ is higher^{7,9} than that of $\text{Ca}(\text{NOTA})^-$, while the complexes of Ca^{2+} with the larger tetraaza tetracarboxylates are all more stable than the corresponding Mg^{2+} complexes.¹⁰ The structure of $\text{Mn}(\text{NOTA})^-$ in solution is apparently similar to the solid-state structures described above, since NMRD studies of the relaxation rate of water protons in the solutions of $\text{Mn}(\text{NOTA})^-$ indicate there are no inner-sphere water molecules.²

The relationship between the structure of these macrocycles (ring size, number of the five- versus six-membered chelate rings)

and the thermodynamic and kinetic stability of their complexes is not well understood despite numerous investigations in this area. We have initiated the preparation and study of other triaza tricarboxylate macrocycles in an effort to further delineate these relationships and report here the acid–base and complexation properties of the new 12-membered-ring ligand 1,5,9-triazacyclododecanetriacetic acid (H_3DOTRA).

Experimental Section

Materials. All chemicals were of analytical grade and were used without further purification. Triazacyclododecane trihydrobromide was synthesized as described elsewhere.¹¹ Solutions of ZnCl_2 , CdCl_2 , MnCl_2 , CaCl_2 , and MgCl_2 were standardized complexometrically. The concentration of the DOTRA solution was also determined by complexometry using a standardized CuCl_2 solution and murexide as an indicator. KOH was standardized by potentiometric titration against potassium hydrogen phthalate¹² and stored under a N_2 atmosphere.

1,5,9-Triazacyclododecane. 1,5,9-Triazacyclododecane trihydrobromide (0.79 g, 1.91 mmol) was dissolved in 10 mL of water and the pH adjusted to 12 or higher using 5 M NaOH. The solution was concentrated to about 5 mL in vacuo and extracted with methylene chloride (3×5 mL). The organic extracts were combined, dried over NaOH pellets, and filtered. The solvent was evaporated in vacuo to leave the free amine as a yellow oil in 89% yield (0.29 g, 1.69 mmol): ^1H NMR (CDCl_3), δ 1.45 (p, 6 H), 2.55 (t, 12 H); ^{13}C NMR (CDCl_3), δ 26.23, 47.43.

1,5,9-Triazacyclododecane-1,5,9-triacetic Acid Trimethyl Ester. 1,5,9-Triazacyclododecane (0.076 g, 0.44 mmol) was dissolved in 5 mL

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of methylene chloride and this solution mixed with 1.5 mL of 1 M NaOH (1.5 mmol). A solution of 0.13 mL of methyl bromoacetate (0.21 g, 1.35 mmol) in 5 mL of methylene chloride was then added dropwise over a period of 1 h, with stirring at room temperature. The mixture was typically stirred overnight. Periods longer than 12 h resulted in partial hydrolysis of the product and its loss from the organic layer. The layers were separated, and the aqueous layer was washed with methylene chloride (3 × 5 mL). The organic portions were combined, dried over NaOH pellets, treated with decolorizing carbon, and filtered through a thin bed of silica gel. The solvent was evaporated in vacuo to give the triester in 80% yield (0.136 g, 0.35 mmol): ¹H NMR (CDCl₃): δ 1.50 (p, 6 H), 2.55 (t, 12 H), 3.16 (s, 6 H), 3.58 (s, 9 H); ¹³C NMR (CDCl₃): δ 21.76, 49.03, 51.19, 55.39, 171.66.

1,5,9-Triazacyclododecane-1,5,9-triacetic Acid Trihydrochloride. The methyl ester (0.136 g, 0.35 mmol) was suspended in water (5 mL) and the pH adjusted to 1 or less with 6 M HCl. The sample was heated at reflux for 12 h, filtered, and concentrated to a volume of 1–2 mL. The pH was adjusted to 5–6 using 2 M NaOH and the solution loaded onto a 2.5 × 30 cm column of Dowex-1 anion-exchange resin (chloride form, 100–200 mesh). The column was washed with 250 mL of water and then eluted with a linear gradient of 0–0.1 M HCl, while the eluant absorbance was monitored at 254 nm. The fraction containing the product was identified by ¹H and/or ¹³C NMR spectroscopy, isolated, concentrated, and freeze-dried. The product was obtained as a white powder in 83% yield. Elemental analysis indicated the trihydrochloride dihydrate: mp 67 °C dec; ¹H NMR (D₂O, pH 0.5, reference HOD at δ 4.80): δ 4.04 (s, 6 H), 3.42 (t, 12 H), 2.24 (p, 6 H); ¹³C NMR (D₂O, pH 1.0, reference *p*-dioxane at δ 67.40): δ 170.40, 56.10, 51.60, 20.30. Anal. Calcd for C₁₅H₂₇N₃O₆·3HCl·2H₂O: C, 36.70; H, 6.98; N, 8.56; Cl, 21.67; O, 26.08. Found: C, 36.13; H, 6.98; N, 8.57; Cl, 21.62; O, 26.70 (by difference).

Potentiometric Measurements. The protonation constants of DOTRA³⁻ were determined by pH potentiometry at 25 °C using an Orion Research Model 701A pH meter and a Cole Parmer combination electrode. Tetramethylammonium chloride (0.1 M) was added to each solution to maintain constant ionic strength. The titrated samples (5–10 mL) were covered by a cyclohexane layer to exclude CO₂. The hydrogen ion concentration was obtained from the measured pH values by the method suggested by Irving et al.¹³ (pK_w = 13.891). The protonation constants of the ligand DOTRA³⁻ (=L³⁻) are defined as follows:

$$K_i = \frac{[H_iL]}{[H_{i-1}L][H^+]} \quad (1)$$

where $i = 1, 2, 3$, or 4.

The first protonation constant K_1 was too high to determine by potentiometry. The titration curve obtained between pH 2 and 10 best fit three protonations, K_2 , K_3 , and K_4 , which were extracted from the data by using a simplex algorithm written in BASIC.¹⁴

The stability constants of Zn(DOTRA)⁻ and Cd(DOTRA)⁻ were determined by potentiometric titration of solutions containing a 1:1 ratio of metal and ligand. Due to the slow formation of Ca(DOTRA)⁻ and Mg(DOTRA)⁻, the stability constants of these complexes were obtained by an "out-of-cell" technique. Six to eight samples (1.5 or 2 mL each) containing DOTRA and Mg(II) (1:1) or DOTRA and Ca(II) (1:2) were prepared, and differing amounts of KOH were added to each sample (between 3 and 4 equiv of KOH per ligand). The samples were stored in closed test tubes covered by a layer of cyclohexane at room temperature, and the pH was measured daily. After 1 week, equilibrium had been established in each solution. The titration points could be fitted by assuming the formation of a single species (1:1). All ML stability constants ($K = [ML]/[M][L]$) were evaluated from the potentiometric data by using a simplex algorithm written in BASIC.

Spectrophotometric Measurements. The first protonation constant of DOTRA³⁻ was determined by spectrophotometry. The UV spectrum of DOTRA³⁻ exhibits a change at pH > 11.5, and the absorbance values (A) become constant in 0.7–1.0 M KOH solutions. With the assumption that the molar absorptivity of the species DOTRA³⁻ (ϵ_L) does not change with the ionic strength, ϵ_L was calculated from the absorbances measured in 0.7 and 1.0 M KOH solutions. Since the spectra do not change between pH 9.5 and 11.5, where the monoprotonated species HDOTRA²⁻ predominates, its molar absorptivity (ϵ_{HL}) was obtained from those measured absorbances. The molar absorptivities of the species HDOTRA²⁻ and DOTRA³⁻ are 915 and 1360 M⁻¹ cm⁻¹, respectively, at 236 nm and 685 and 1100 M⁻¹ cm⁻¹, respectively, at 240 nm. Changing the concentration

of KOH in the samples but keeping the sum of the concentration of KOH and tetramethylammonium chloride constant (0.1 M) allows the concentrations of the species HDOTRA²⁻ and DOTRA³⁻ to be calculated using the following equations:

$$A = \epsilon_{HL}[HL] + \epsilon_L[L] \quad (2)$$

$$C_L = [HL] + [L] \quad (3)$$

The combination of eq 2 and 3 gives for $[L]$

$$[L] = \frac{A - \epsilon_{HL}C_L}{\epsilon_L - \epsilon_{HL}} \quad (4)$$

If $[L]$ is known, the value of $[HL]$ and the protonation constant K_1 may be calculated. Since the concentration of DOTRA was very low (2×10^{-4} M) compared to the KOH concentration (0.01–0.1 M), the very small change in the concentration of OH⁻ ions due to deprotonation of HDOTRA²⁻ was neglected.

Spectrophotometric measurements were carried out on a Cary 219 spectrophotometer at 25 °C using 1-cm cells.

Relaxation Rate Measurements. The formation of the Mn(DOTRA)⁻ complex may be conveniently followed by measuring the spin-lattice relaxation rate ($1/T_1$) of the water protons, which decreases considerably upon complexation of free Mn²⁺ by DOTRA³⁻. The relaxation rate of water protons ($1/T_1$) in the presence of Mn²⁺ and DOTRA³⁻, assuming the formation of a 1:1 complex, may be expressed as follows:

$$1/T_1 = R_{Mn}[Mn] + R_{MnL}[MnL] + 1/T_{10} \quad (5)$$

where $1/T_{10}$ is the relaxation rate in the absence of Mn²⁺ and R_{Mn} and R_{MnL} are the relaxivities (proton relaxation rate/metal ion or metal chelate concentrations¹⁵) of Mn²⁺ or Mn(DOTRA)⁻, respectively. R_{MnL} may be determined by measuring the relaxation rate of a fully formed Mn(DOTRA)⁻ complex (complexation is complete at pH > 6.3 in solutions containing 1:1 metal to ligand after several hours at room temperature). At pH values where free Mn²⁺, free DOTRA, and Mn(DOTRA)⁻ are all present, the concentration of Mn(DOTRA)⁻ may be determined using the expression

$$[MnL] = \frac{R_{Mn}[Mn]_t - 1/T_1'}{R_{Mn} - R_{MnL}} \quad (6)$$

where $1/T_1' = 1/T_1 - 1/T_{10}$ and $[Mn]_t = [Mn] + [MnL]$. Thus, a series of conditional stability constants for Mn(DOTRA)⁻ may be determined from relaxivity measurements on samples at equilibrium at various pH values. The thermodynamic stability constant of Mn(DOTRA)⁻ was obtained by multiplying each conditional constant by α_L , where $\alpha_L = 1 + K_1[H^+] + K_1K_2[H^+]^2 + \dots$ ¹⁴. The values of α_L are evaluated using the known protonation constants of DOTRA³⁻.

Water proton relaxation rates were determined on 0.1-mL samples by the inversion recovery method using a Spin-Lock pulsed NMR operating at 40 MHz.

NMR Spectral Measurements. Solutions of the ligands or of the ligand/metal ion mixtures were made up in D₂O (99.7% from Sigma) and the pH was adjusted with DCl or CO₂-free NaOD (Sigma). The NMR pH titrations of H₃DOTRA were carried out in a NMR tube using CO₂-free KOD (made from 99.7% D₂O and potassium metal) and were corrected for a deuterium isotope effect.

¹H and broad-band proton-decoupled ¹³C NMR spectra were recorded on a JEOL JNM-FX 200 or a General Electric GN-500 spectrometer. Proton and ¹³C shifts were referenced to TMS using *tert*-butyl alcohol and dioxane, respectively, as internal standards. Probe temperatures were accurate to ±0.5 °C. Spectral simulations were carried out using software on the GN-500 spectrometer. Splitting patterns of the proton spectra in the synthesis section are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; p, quintet; m, multiplet; br, broad peak.

Results and Discussion

Protonation Constants. The first protonation constant of the nine-, ten-, eleven- and twelve-membered-ring triazacycloalkanes increases with increasing ring size, indicating an enhancement in the basicity of the ring nitrogens.¹⁶ The tricarboxylate derivatives of these ligands show a similar behavior, which is reflected in the very high value of the first protonation constant of DOTRA³⁻ in comparison to that of NOTA³⁻. The log K_1 values obtained by spectrophotometry at different KOH concentrations and wavelengths agree well, as summarized in Table I. The

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Table I. First Protonation Constant of DOTRA Obtained by Spectrophotometry ($I = 0.1$ M, 25 °C)

C_{OH}, M	$\log K_1$		C_{OH}, M	$\log K_1$	
	236 nm	240 nm		236 nm	240 nm
0.01	12.85	12.86	0.05	12.77	12.79
0.02	12.84	12.80	0.07	12.84	12.85
0.03	12.69	12.63	0.10	12.75	12.75

$$\log K_1(av) = 12.8 \pm 0.1$$

Table II. Protonation Constants of [9]ane-N₃, [12]ane-N₃, NOTA, and DOTRA ($I = 0.1$ M, 25 °C)

	[9]ane-N ₃ ^a	NOTA ^b	[12]ane-N ₃ ^a	DOTRA ^c
$\log K_1$	10.42	11.41	12.60	12.8
$\log K_2$	6.42	5.74	7.57	7.55
$\log K_3$	low	3.16	2.41	3.65
$\log K_4$		1.71		2.1

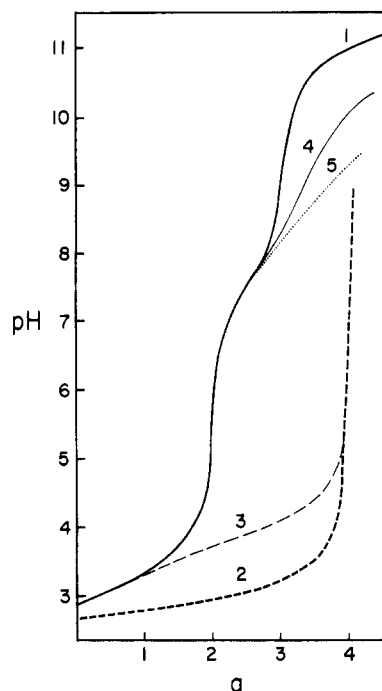
^a From ref 16. ^b From ref 7. ^c This work.

average value ($\log K_1 = 12.8$) is quite similar to the first protonation constant of [12]ane-N₃ ($\log K_1 = 12.6^{16}$). The protonation constants of DOTRA³⁻ obtained by spectrophotometry and pH potentiometry are shown in Table II, where the protonation constants of [12]ane-N₃, as well as [9]ane-N₃ and NOTA³⁻ are also presented. From a comparison of these data it can be seen that all protonation constants of the 12-membered-ring ligands are higher than those of the 9-membered-ring ligands, where the electrostatic repulsion between the protonated nitrogens is greater.

The first protonation constant of [12]ane-N₃ and DOTRA is also much higher than the $\log K_1$ values of the noncyclic triamines. Other investigators have proposed that a strong hydrogen bond can be formed between two nitrogens of [12]ane-N₃ and that the cavity size of the 12-membered ring is particularly suitable to include a H₃O⁺ molecule.¹⁶ The high $\log K_1$ value of DOTRA³⁻ measured here can be interpreted similarly.

The protonation of the ligands can be followed by ¹H NMR spectroscopy, since the protonation of donor atoms generally results in a deshielding of the nonlabile hydrogens and changes in chemical shift can indicate the site of protonation.¹⁷ The ¹H NMR spectra of [12]ane-N₃ and DOTRA have been measured over a broad interval of pH.²⁴ The chemical shifts of the propylene protons of [12]ane-N₃ change considerably over the pH intervals 12–13.5, 6.5–8.5 and 1.7–3.3, where protonations take place. The pH at the center of these chemical shift intervals agrees reasonably with the protonation constants of [12]ane-N₃. The chemical shifts of the propylene and acetate methylene protons of DOTRA change similarly above pH 5. These data indicate that the first two proton sites, i.e., formation of HDOTRA²⁻ and H₂DOTRA⁻, occur at two of the macrocyclic nitrogens, similar to that observed for NOTA.¹⁷ Between pH 1.5 and 4.5 the chemical shift of acetate methylene protons changes, while that of the propylene protons shows practically no change. This indicates that one or two carboxylates are protonated over this pH range. In stronger acid, i.e., below pH 1.5, protonation of the acetate groups and the third nitrogen atom takes place, as inferred from the shift of both the propylene and acetate proton resonances.

Complexation of DOTRA³⁻. The complex-forming properties of DOTRA³⁻ are strongly influenced by the very high value of the first protonation constant as well as by the conformational requirements of the 12-membered ring. Presumably, these properties account for much slower complexation reactions of DOTRA³⁻ with metal ions than those of the cyclononane analogue NOTA³⁻ and the fact that Ca²⁺ and Mg²⁺ complexes with DOTRA³⁻ are formed only at high pH. This behavior limits the study of metal ion–DOTRA complexes to those ions that form relatively strong bonds with nitrogen donors or that do not hydrolyze below about pH 10. The formation of Zn(DOTRA)⁻ and Cd(DOTRA)⁻ could be studied by usual pH potentiometric techniques, although the Cd(DOTRA)⁻ system reached equilib-

**Figure 1.** Potentiometric titration curves of 1 mM DOTRA (1) and those obtained in the presence of equivalent amounts of Zn²⁺ (2), Cd²⁺ (3), Ca²⁺ (4), and Mg²⁺ (5) (α = ratio of KOH added to total ligand).**Table III.** Stability Constants of NOTA and DOTRA Complexes ($I = 0.1$ M, 25 °C)

ion	$\log K_{st}$		ion	$\log K_{st}$	
	NOTA	DOTRA ^b		NOTA	DOTRA ^b
Zn ²⁺	18.3 ^a	19.0	Mg ²⁺	9.69, ^c 8.93 ^d	7.1
Cd ²⁺	16.0 ^a	15.7	Ca ²⁺	8.92, ^c 8.81 ^d	6.0
Mn ²⁺	14.3, ^a 14.9 ^b	12.8			

^a From ref 3. ^b This work. ^c From ref 9. ^d From ref 7.

rium three to four times more slowly than the Zn(DOTRA)⁻ system. The formation of Ca(DOTRA)⁻ and Mg(DOTRA)⁻ was much slower, and their stability constants were determined using an "out-of-cell" technique. The titration curves obtained in the presence of Ca²⁺ or Mg²⁺ differed from that of the ligand only when the last 1 equiv of base was added; i.e., only the monoprotonated form, HDOTRA²⁻, was able to react with the Ca²⁺ or Mg²⁺ ions. The titration curves are shown in Figure 1, and the stability constants calculated from the data are presented in Table III.

The Mn(DOTRA)⁻ system reached equilibrium during 1–5 h, depending upon the pH of the sample. The use of water proton relaxation rates to determine stability constants gives reliable results only when the two species that are present (i.e., Mn²⁺ and Mn(DOTRA)⁻) have reasonably different relaxivities. These conditions are realized by the Mn(DOTRA)⁻ system. In order to compare the value of stability constant obtained by relaxivity measurement with a value determined by other methods, the stability constant of Mn(NOTA)⁻ was also determined. This value ($\log K = 14.9$; Tables III and IV) agrees quite well with the value obtained by polarography ($\log K = 14.3^{31}$), particularly if the different protonation constants used for the calculations are also taken into account. The stability constants of Mn(NOTA)⁻ and Mn(DOTRA)⁻ obtained at different metal to ligand ratios and pH are shown in Table IV. The agreement of these $\log K$ values demonstrates the feasibility of this method and that the assumptions involved (1:1 complexes only) are valid. The determination of stability constants of Mn(II) complexes by relaxivity measurements is particularly advantageous in those cases when limited ligand is available, since the determination can be carried out on as little as 1–2 mL of a 1 mM solution.

A comparison of the data presented in Table III shows that the stability constants of the complexes of Zn(II) and Cd(II)

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Table IV. Stability Constants of $\text{Mn}(\text{NOTA})^-$ and $\text{Mn}(\text{DOTRA})^-$ Obtained by Proton Relaxation Rate Measurements ($I = 0.1 \text{ M}$, 25°C)

expt no.	$\text{Mn}(\text{NOTA})^-$				$\text{Mn}(\text{DOTRA})^-$			
	$C_L/C_{\text{Mn}} = 1.2$		$C_L/C_{\text{Mn}} = 2.0$		$C_L/C_{\text{Mn}} = 1.2$		$C_L/C_{\text{Mn}} = 2.0$	
	pH	$\log K_{\text{st}}$	pH	$\log K_{\text{st}}$	pH	$\log K_{\text{st}}$	pH	$\log K_{\text{st}}$
1	3.74	14.94	3.37	14.71	6.30	12.80	5.99	12.87
2	3.54	14.97	3.17	14.82	6.19	12.97	5.91	12.64
3	3.45	15.01	3.06	14.81	6.10	12.83	5.76	12.72
4	3.36	15.00	2.92	14.98	6.01	12.82	5.64	12.76
5	3.26	14.96	2.83	15.01	5.91	12.79	5.52	12.75
6	3.15	14.93	2.71	15.03	5.83	12.79	5.37	12.72
7	3.05	14.94	2.57	14.90	5.54	12.81	5.25	12.84
8	2.94	15.01	2.42	14.97	5.48	12.83	5.11	12.92
	av $\log K_{\text{st}}$: 14.9 ± 0.1				av $\log K_{\text{st}}$: 12.8 ± 0.1			

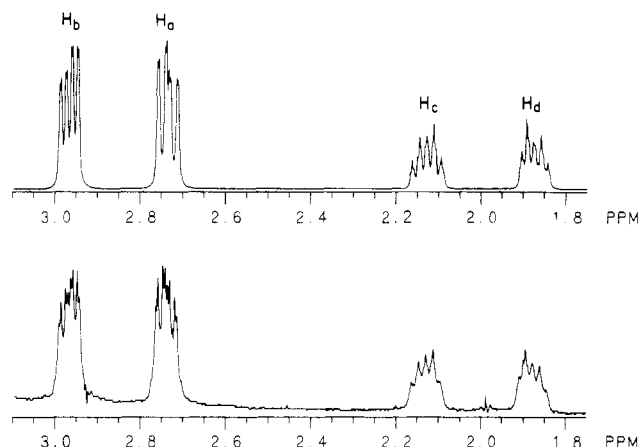
formed with the NOTA^{3-} and DOTRA^{3-} are quite similar. However, the complexes of NOTA^{3-} formed with $\text{Mn}(\text{II})$, $\text{Ca}(\text{II})$, and $\text{Mg}(\text{II})$ are more stable than the corresponding DOTRA^{3-} complexes. The interpretation of these results is not easy, particularly if we take into account that the complex of $\text{Zn}(\text{II})$ (as well as $\text{Cu}(\text{II})$ and $\text{Ni}(\text{II})$) formed with [9]ane- N_3 is much more stable than its complex with [12]ane- N_3 .¹⁶

A consideration of the complexation tendencies of the metal ions provides a starting point for the interpretation of these experimental findings. It is known that $\text{Zn}(\text{II})$ and $\text{Cd}(\text{II})$ tend to form complexes with nitrogen donor ligands in aqueous solution, while $\text{Mn}(\text{II})$, $\text{Ca}(\text{II})$, and $\text{Mg}(\text{II})$ show less tendency to do so. Thus, the near equality of the stability constants of the NOTA^{3-} and DOTRA^{3-} complexes of $\text{Zn}(\text{II})$ and $\text{Cd}(\text{II})$ suggests that the metal–nitrogen bonds play an important role. The metal–nitrogen bond distances in the DOTRA^{3-} and NOTA^{3-} complexes may be similar only if the Zn^{2+} or Cd^{2+} ions occupy a position closer to the plane of the three nitrogens in the DOTRA^{3-} complex. This difference would also allow the carboxylate arms to move closer to the central ion in the $\text{Zn}(\text{DOTRA})^-$ and $\text{Cd}(\text{DOTRA})^-$ complexes, thereby enhancing their stability over that expected solely on the basis of changing from five-membered chelate rings (NOTA complexes) to six-membered chelate rings (DOTRA complexes). $\text{Mn}(\text{II})$, $\text{Ca}(\text{II})$, and $\text{Mg}(\text{II})$, however, interact less favorably with the nitrogen donors, and consequently these same structural changes may not be possible in these complexes. This would leave all of the metal ion–donor atom distances longer, and as a consequence, the corresponding stability constants are lower for the DOTRA complexes than the NOTA complexes, as expected. We are currently attempting to crystallize these complexes to confirm this hypothesis.

The coordination cage formed by the donor atoms of DOTRA seems not to be much larger than that of NOTA in spite of its larger, 12-membered ring. The stability order of the Mg^{2+} and Ca^{2+} complexes surprisingly agrees with the order found for the NOTA complexes,^{6,9} since $\text{Mg}(\text{DOTRA})^-$ is more stable than $\text{Ca}(\text{DOTRA})^-$.

In general, either the stability constants of the respective metal ion– NOTA and – DOTRA complexes are quite similar or the complexes with the larger macrocyclic ligand are somewhat less stable. This indicates that replacement of the three five-member chelate rings in NOTA by six-member rings in DOTRA does not result in a very significant drop in the complex stability. However, similar structural changes in the tetraaza tetracarboxylate macrocycles results in a very significant decrease in complex stability even when only two five-member chelate rings are replaced by six-member rings, i.e., DOTA versus TETA .^{7,18}

Proton NMR spectroscopy was also used to study the interaction of DOTRA with various diamagnetic metal ions. The proton NMR spectra of the Zn^{2+} , Cd^{2+} , and Mg^{2+} complexes indicate strong complex formation in 1:1 stoichiometry. Figure 2 shows the 500-MHz proton spectrum of the $\text{Cd}(\text{DOTRA})^-$ complex together with its simulated spectrum. The proton NMR spectra of $\text{Zn}(\text{DOTRA})^-$ and $\text{Mg}(\text{DOTRA})^-$ are nearly identical except

**Figure 2.** 500-MHz ^1H spectra of $\text{Cd}(\text{DOTRA})^-$ at pH 5.2: (bottom) experimental; (top) simulated. The acetate singlet at 3.24 ppm is not shown.**Table V.** NMR Chemical Shifts, Coupling Constants, and Dihedral Angles for the Ring Protons in the DOTRA Complexes

	Zn^{2+}	Cd^{2+}	Mg^{2+}		Zn^{2+}	Cd^{2+}	Mg^{2+}
Chemical Shifts (ppm)							
δ_a	2.84	2.74	2.65	δ_c	2.08	2.13	1.93
δ_b	3.11	2.97	2.91	δ_d	1.83	1.88	1.72
Vicinal Coupling Constants (Hz)							
J_{ac}	8.27	8.84	8.29	J_{bc}	1.62	2.20	1.50
J_{ad}	1.11	1.94	1.09	J_{bd}	7.75	6.90	7.24
Geminal Coupling Constants (Hz)							
J_{ab}	13.54	13.70	13.37	J_{cd}	15.79	16.94	15.68
Dihedral Angles (deg)							
α	33	29	32	γ	62	79	63
β	36	41	39				

for small differences in chemical shifts and coupling constants (Table V). In each complex, the methylene protons a, b, c, and d of the propylenediamine bridges are nonequivalent, yielding an $\text{A}_2\text{B}_2\text{XY}$ type spin pattern. These proton resonances were assigned on the basis of their relative intensities and the chemical shift effects expected for a six-membered chelate ring locked in a pseudo chair conformation (see Figure 3). In this conformation the equatorial protons b and c are expected to be displaced to higher frequency relative to the axial protons a and d in the same CH_2 fragment as a result of the shielding behavior of the C–C single bonds, as found for cyclohexane- d_{11} at very low temperature,¹⁹ and the electric field effect caused by the metal ion on the polarization of the C–H bonds.^{20,21}

The proton spectra of $\text{Zn}(\text{DOTRA})^-$ and $\text{Cd}(\text{DOTRA})^-$ do not change significantly with temperature between 25 and 80°C ,

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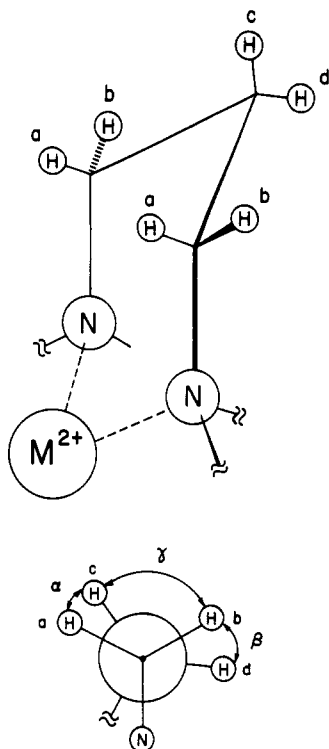


Figure 3. Approximate conformation of the chelate rings in the $M(\text{DOTRA})^-$ complexes.

showing that the three six-membered chelate rings in these complexes are quite rigid. Some information about their ring conformations may be obtained from calculations of the dihedral angles between the ring C-H bonds (shown as a Newman projection in Figure 3) by using the experimental vicinal coupling constants and an appropriate Karplus equation for hydrocarbons, $^3J_{\text{HH}} = 7 - \cos \phi + 5 \cos 2\phi$.²⁰ This equation does not take into account the electronegativity effects of the ring nitrogens, but it can be used for a qualitative comparison of the three complexes. The calculated dihedral angles, summarized in Table V, indicate that the different ionic radius of Cd^{2+} (0.97 Å) versus Zn^{2+} (0.74 Å) or Mg^{2+} (0.65 Å) has only a small effect upon the conformation of the six-membered chelate rings. The Cd^{2+} ion being larger than Zn^{2+} or Mg^{2+} forces protons a and b further away from the ring pseudoplane and increases the dihedral angle between protons b and c. J_{ab} is also smaller than J_{cd} , as expected, due to the α effect of the electronegative nitrogen on the $\text{CH}_2(\text{a,b})$ fragment.^{21,22} A quantitative account of the α -electronegativity effect of the ring nitrogens on the vicinal proton coupling constants is quite difficult, as this effect depends on the electronegativity of the heteroatom and on its orientation relative to the C-H bonds.²³ Qualitatively, one would predict a small positive contribution to J_{ac} and J_{bc} and a small negative contribution to J_{ad} and J_{bd} . The fact that J_{ac}

and J_{bd} are much larger than J_{ad} and J_{bc} is certainly a reflection of ring distortion from a pure eclipsed conformation. Although $J_{\text{ac}} > J_{\text{bd}}$ and $J_{\text{bc}} > J_{\text{ad}}$ for each of the three diamagnetic complexes examined, the calculated dihedral angles (Table V) are not entirely consistent with that expected on the basis of metal ion size variations. This likely reflects electronegativity effects of the nitrogen heteroatoms that cannot be quantitatively predicted in the three complexes.

The proton NMR spectrum of a solution containing $\text{Ca}(\text{DOTRA})^-$ at high pH where the complex should be fully formed looks very much like the spectrum of the free ligand at high pH but with slightly broadened resonances. This indicates $\text{Ca}(\text{DOTRA})^-$ is considerably more labile than the other complexes studied and Ca^{2+} is not chelated strongly enough to make the propylenediamine rings rigid as in the other complexes.

Conclusions

An increase in the ring size of the triaza tricarboxylate macrocyclic chelates from 9 (NOTA) to 12 (DOTRA) affects both the protonation and complexation properties of these chelates. The protonation constants increase significantly with an increase in size of the macrocycle, apparently due to a decrease in electrostatic repulsion between the protonated nitrogen atoms. The stability of the complexes formed by DOTRA with Zn^{2+} and Cd^{2+} are quite similar to their respective complexes with NOTA, while the Mn^{2+} , Ca^{2+} , and Mg^{2+} complexes with DOTRA are less stable than their respective NOTA complexes. This is probably the consequence of the relatively small coordination cage formed by the donor atoms of NOTA and DOTRA, which is reflected in the higher stability constants of both $\text{Mg}(\text{NOTA})^-$ and $\text{Mg}(\text{DOTRA})^-$ relative to the respective $\text{Ca}(\text{NOTA})^-$ and $\text{Ca}(\text{DOTRA})^-$ complexes.

These differences are a result of a combination of factors including metal ion size, macrocyclic chelate hole size, chelate ring size, and the degree of metal ion-donor atom covalency. In the Zn^{2+} and Cd^{2+} complexes, the more favorable macrocyclic ring size of DOTRA versus NOTA for these ions is offset by the less favorable six-membered chelate rings formed in the DOTRA complexes versus the five-membered chelate rings formed in the NOTA complexes. Although this same factor should be present in the Mn^{2+} , Mg^{2+} , and Ca^{2+} complexes, these ions show less tendency to form stable covalent bonds with nitrogen donor atoms of either DOTRA or NOTA, and hence these energy terms are less important in determining the stability of these complexes. This is best seen by comparing the thermodynamic stability and kinetic lability of the Cd^{2+} and Ca^{2+} complexes with DOTRA. These ions have similar ionic radii, and both complexes obviously form three six-membered ring chelate structures. However, the more covalent Cd^{2+} -N bonds of $\text{Cd}(\text{DOTRA})^-$ yield a complex that is both considerably more stable and kinetically inert than the $\text{Ca}(\text{DOTRA})^-$ complex.

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Registry No. DOTRA, 106415-34-3; DOTRA·3HCl, 123726-20-5; DOTRA (trimethyl ester), 123726-21-6; Zn, 7440-66-6; Cd, 7440-43-9; Ca, 7440-70-2; Mg, 7439-95-4; Mn, 7439-96-5; 1,5,9-triazacyclodecane, 294-80-4.

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